Result

Our wet lab experiment is consisted of three major parts, construction and testing of our circuit, testing of mlrD and mlrA, testing of riboswitches. Photos of electrophoresis[1] and results of sequencing will be listed as basic results of construction and results of HPLC and fluorescence measurement will work as efficiency verification of our project.

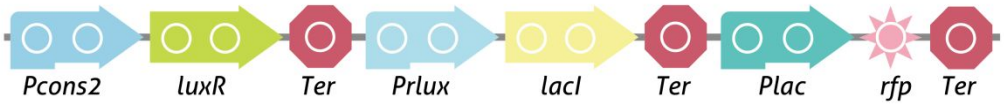
In actual experiment, our project is composed of three sections, the detector, the positive feedback circuit, the suicide circuit which are all used to test our design in experiment. In the process of construction, we first construct small devices from parts provided in the kits and then put all these devices together step by step.

Part 1:Circuit

1. Detector circuit

The detector circuit is used to measure concentration of AHL and present fluorescence at certain concentration. It is made up of two plasmids.

(1)Plasmid 1: Pcons-rbs-luxR-T-Prlux-rbs-lacI-T-Plac-rbs-rfp-T



A. division 1 : Pcons2-rbs-luxR-T

Figure[2]: From left to right, Marker(DL10000), Pcons2, Pcons2-luxR, Pconss-luxR-Ter[3]

Sequencing results:

We have ligated and sequenced this composite several times. Although the results of electrophoresis are identical to our expectation, the results of

[1] For specific procedures, please refer to the protocols.

[2] The actual photos of electrophoresis are listed in the attachment, while the results for each division maybe combination of results from different electrophoresis. However, we make sure that the marker matches while splicing.

[3] Primers used in PCR are VF2 and VR, and following electrophoresis results are similar. .

sequencing always show that the where there is luxR is not it[4]. However, the same luxR in other composites meet our expectations. So, we suspect the authenticity of sequencing in this composite. We will verify luxR in our testing of function.

B. division 2: Prlux-rbs-lacI-T

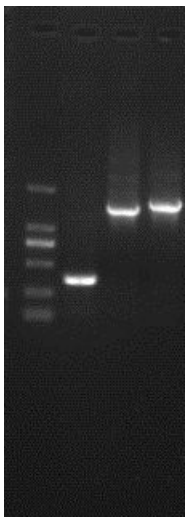


Figure [5]: From left to right M：Marker（DL2000）, a: Prlux, Prlux-rbs-lacI, Prlux-rbs-lacI-T. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

Sequencing result[6]:

CTATAAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAAATCCT
TAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTAGAAAA
GAGGAGAAATTAAGCATGGTGAATGTGAAACCAGTAACGTTATACGATG
TCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGA
ACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCG
GCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTG
GCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGC
CCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGA
TCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCG
TCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTC
AGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTG
TGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGA
CCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGA
CTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCT
GTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTG
GCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACG
GGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAAT

[4] It is actually something there, however it doesn't match luxR.
[5] To make the results clear, we get rid of band of other parts in this photo. The whole photo of electrophoresis will be listed later.
[6] Primers used in sequence is VF2 and VR.

GCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCA
GATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGC
GTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGC
TCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGC
TGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAG
GCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAA
AACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGG
CCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCG
GGCAGGCTGCAAACGACGAAAACTACGCTTTAGTAGCTTAATAACTCTG
ATAGTGCTAGTGTAGATCTCTACTAGTAGCGGCCGCTGCAGTCCGGCA
AAAAAGGGCAAGGTGTCACCACCCTGCCCTTTTTCTTTAAAACCGAAA
AGATTACTTCGCGTTATGCAGGCTTCCTCGCTCACTGACTCGCTGCGC
TCGGTCGT

C. Division 3: Plac-rbs-rfp-T

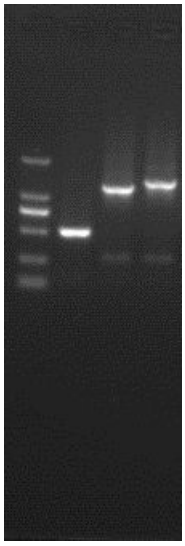
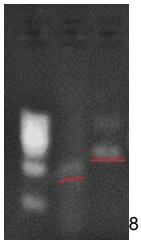


Figure : From left to right Marker（DL2000）, Plac, Plac-rbs-rfp, Plac-rbs-rfp-T. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

Sequencing results:

The sequencing results is always wrong in this composite, but we actually saw the red fluorescence of colonies[7]. So, we stop the tedious job of sequencing and assume this composite is right.

D. Linking of Pcons2-rbs-luxR-T and Prlux-rbs-lacI-T

 [8]

[7] Shown in the following passages

[8] For the reason of additional sequence up and down the aim sequence, the result line is a little longer.

Figure: From left to right, Marker(1Kb), Pcons2-rbs-luxR-Ter, Pcons2-rbs-luxR-Ter-Prlux-rbs-lacI-Ter

E. Linking of Pcons2-rbs-luxR-T-Prlux-rbs-lacI-T and Plac-rbs-rfp-T

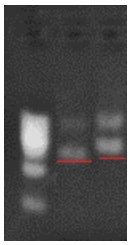
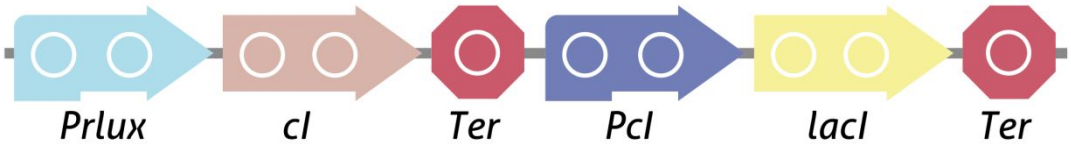


Figure: From left to right, Marker(1Kb), Pcons2-rbs-luxR-Ter-Prlux-rbs-lacI-Ter, Pcons2-rbs-luxR-Ter-Prlux-rbs-lacI-Ter-Plac-rbs-rfp-Ter

(2) Plasmid 2: Prlux-rbs-cI-T-PcI-rbs-lacI-T



A. Division 1: Prlux-rbs-cI-T

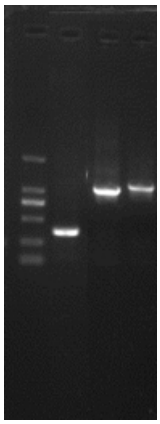


Figure: From left to right, Marker （DL2000）, Prlux, Prlux-rbs-cI, Prlux-rbs-cI-T. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

B. Division 2: PcI-rbs-lacI-T

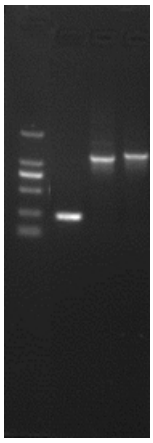


Figure: From left to right, Marker （DL2000）, PcI, PcI-rbs-lacI, PcI-rbs-lacI-T. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

C. Division 3: Prlux-rbs-cI-T-PcI-rbs-lacI-T

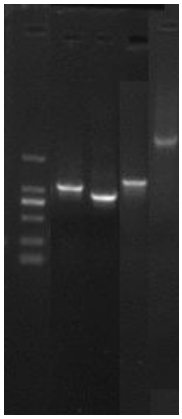


Figure: From left to right, Marker （DL2000）, Prlux-rbs-cI-T, PcI-rbs-lacI-T, Prlux-rbs-cI-T-PcI-rbs-lacI-T. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

Sequencing results:

This part is rather long, and even one walking can't go through the whole sequence. So, we list three part of our sequence results.

VF2:

CCTATAAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAA
AAATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGC
TTCTAGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTT
TGTTATAGTCGAATAAATACTAGAAAAGAGGAGAAATTAAGCATGAG
CACAAAAAAGAAACCATTAACACAAGAGCAGCTTGAGGACGCACG
TCGCCTTAAAGCAATTTATGAAAAAAAGAAAAATGAACTTGGCTTAT
CCCAGGAATCTGTCGCAGACAAGATGGGGATGGGGCAGTCAGGC
GTTGGTGCTTTATTTAATGGCATCAATGCATTAAATGCTTATAACGC
CGCATTGCTTGCAAAAATTCTCAAAGTTAGCGTTGAAGAATTTAGC
CCTTCAATCGCCAGAGAAATCTACGAGATGTATGAAGCGGTTAGTA
TGCAGCCGTCACTTAGAAGTGAGTATGAGTACCCTGTTTTTTCTCA

TGTTCAGGCAGGGATGTTCTCACCTGAGCTTAGAACCTTTACCAAA
GGTGATGCGGAGAGATGGGTAAGCACAACCAAAAAAGCCAGTGAT
TCTGCATTCTGGCTTGAGGTTGAAGGTAATTCCATGACCGCACCAA
CAGGCTCCAAGCCAAGCTTTCCTGACGGAATGTTAATTCTCGTTGA
CCCTGAGCAGGCTGTTGAGCCAGGTGATTTCTGCATAGCCAGACT
TGGGGGTGATGAGTTTACCTTCAAGAAACTGATCAGGGATAGCGG
TCAGGTGTTTTTACAACCACTAAACCCACAGTACCCAATGATCCCA
TGCAATGAGAGTTGTTCCGTTGTGGGGAAAGTTATCGCTAGTCAGT
GGCCTGAAGAGACGTTTGGCGCTGCAaACGACgAAAACTACGCTT
TAGTAGCTT

VR:

ACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCCTGCAT
AACGCGAAGTAATCTTTTCGGTTTTAAAGAAAAAGGGCAGGGTGG
TGACACCTTGCCCTTTTTTGCCGGACTGCAGCGGCCGCACTAGTG
AATTCTATAAACGCAGAAAGGCCCACCCGAAGGTGAGCCAGTGTG
ACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAAAACGAAA
GGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCT
CTAGTAGAGATCTACACTAGCACTATCAGAGTTATTAAGCTACTAAA
GCGTAGTTTTCGTCGTTTGCAGCCTGCCCGCTTTCCAGTCGGGAA
ACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGA
GAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCACC
AGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTG
AGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGC
GAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCT
GTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACG
C

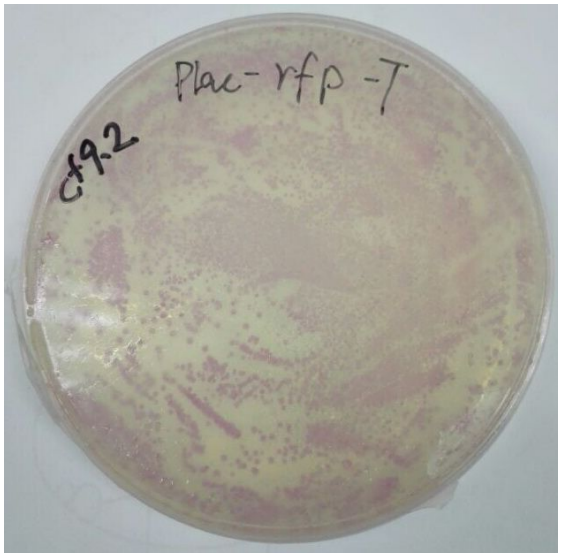
Walking(middle):

TTACCTTCAaGAaACTGATCAGGGATAGCGGTCAGGTGTTTTT
ACAACCACTAAACCCACAGTACCCAATGATCCCATGCAATGAGAGT
TGTTCCGTTGTGGGGAAAGTTATCGCTAGTCAGTGGCCTGAAGAG
ACGTTTGGCGCTGCAAACGACGAAAACTACGCTTTAGTAGCTTAAT
AACGCTGATAGTGCTAGTGTAGATCGCTAATAAACTAGAGCCAGGC
ATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT
TATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGC
TCACCTTCGGGTGGGCCTTTCTGCGTTTATAGAATTCACTAGAGTA
ACACCGTGCGTGTTGACTATTTTACCTCTGGCGGTGATAATGGTTG
CTACTAGATGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCA
GAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAAC
CAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGC
GGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACA
ACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCA
GTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAAT
CTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTA
GAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCT

TCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGA
TGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCC
GGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTA
TTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGG
TCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAA
GTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGgCATAAATATCT
CACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTG
AGGTGCATGTCGATTTCACAATCATGCAATGCTGAATGGAGGCATC
GTT

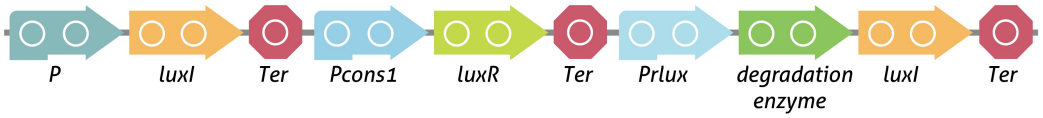
(3) Testing of function

A. The test of function of Plac-rfp-Ter shows that without lacI, rfp was expressed.



2. Positive feedback circuit.

(1) Construction of plasmid



Positive feedback system is consisted of four similar plasmids to test the efficiency of positive feedback and compare with results of modeling.

A. Division 1: Pcons2-rbs-lacI-T

Figure : From left to right, Marker (DL10000), Pcons2, Pcons2-lacI, Pcons-lacI-Ter.

Sequencing results:

CTATAAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAAAT
CCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTAGA
GACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTC
GAATAAATACTAGAAAAGAGGAGAAATTAAGCATGGTGAATGTGAAACC
AGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACC
GTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCG
GGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACC
GCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTT
GCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGC
GATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGAT
GGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATC
TTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATG
ACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGT
TATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCC
CATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGG
TCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGC
GCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATT
CAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTT
TCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGAT
GCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTA
CCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATAC
GACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCA
AACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTG
CAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGT
CTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGC
CTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGT
TTCCCGACTGGAAAGCGGGCAGGCTGCAAACGACGAAAACTACGCTT
TAGTAGCTTAATAACTCTGATAGTGCTAGTGTAGATCTCTAATAAACTAGA
GCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTT
CGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTG

GCTCACCTTCGGGTGGGCCTTTCTGCGTTTATAGAATTCACTAGTGCG
GCCGCTGCAGTCCGGCAAAAAAGGGCAAGGTGTCACCACCCTGCCCT
TTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCAGGCTTCCTCGCT
CACTGACTCGCTGCGCTCGGTCGT

B. Division 2: Plac-rbs-luxI-T

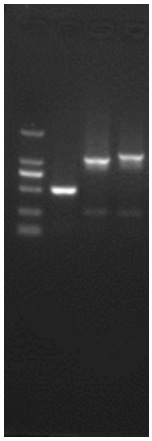


Figure: From left to right, Marker（DL2000）, Plac, Plac-rbs-luxI, Plac-rbs-luxI-T. The substances of electrophoresis are made from PCR of plasmid with VF2 and VR as their primers.

Sequencing results:

ACCTATAaAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAA
ATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTT
CTAGAGCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTC
ATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCA
GTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACC
CCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATT
GTGAGCGGATAACAATTTCACACATACTAGAGAAAGAGGAGAAATT
AAGCATGACTATAATGATAAAAAAATCGGATTTTTTGGCAATTCCATC
GGAGGAGTATAAAGGTATTCTAAGTCTTCGTTATCAAGTGTTTAAGC
AAAGACTTGAGTGGGACTTAGTTGTAGAAAATAACCTTGAATCAGA
TGAGTATGATAACTCAAATGCAGAATATATTTATGCTTGTGATGATAC
TGAAAATGTAAGTGGATGCTGGCGTTTATTACCTACAACAGGTGATT
ATATGCTGAAAAGTGTTTTTCCTGAATTGCTTGGTCAACAGAGTGC
TCCCAAAGATCCTAATATAGTCGAATTAAGTCGTTTTGCTGTAGGTA
AAAATAGCTCAAAGATAAATAACTCTGCTAGTGAAATTACAATGAAA
CTATTTGAAGCTATATATAAACACGCTGTTAGTCAAGGTATTACAGAA
TATGTAACAGTAACATCAACAGCAATAGAGCGATTTTTAAAGCGTAT
TAAAGTTCCTTGTCATCGTATTGGAGACAAAGAAATTCATGTATTAG
GTGATACTAAATCGGTTGTATTGTCTATGCCTATTAATGAACAGTTTA
AAAAAGCAGTCTTAAATGCTGCAAACGACGAAAACTACGCTTTAGT
AGCTTAATAACTCTGATAGTGCTAGTGTAGATCTCTAATAATACTAGA
GCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCC
TTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCACTCGGTCA
CACTGAGAACTAGTAGCGGCCGCTGCAGTCCGGCAAAAAAGGGC

AAGGTGTCACCACCCTGCCCTTTTTCTTTAAAACCGAAAAGATTAC
TTCGCGTTATGCAGGCTTCCTCGCTCACTGACTCGCTGCGCTCGG
TCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAA
TACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGT
GAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCG
TTGCTGGCGTTTTTCCACAGGCTCCGCCCCCCTGACGAGCATCAC
AAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAgGACTA
TAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT
CCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTC
CCTTCGGGAAGCGT

C. Division 3: Pcons-rbs-luxR-T

The figure is same with the one in the Detector circuit division 1.

Sequencing results:

The same with that in the Detector circuit division 1.

D. Division 4: Prlux-rbs-rfp-T, Prlux-rfp-luxI-T, Prlux-rfp-luxR-T, Prlux-rfp-luxI-luxR-T

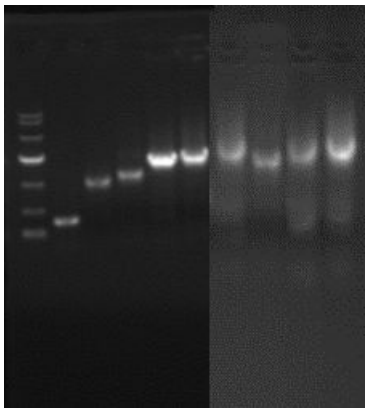


Figure: From left to right, Marker(DL10000), Prlux, Prlux-rbs-rfp, Prlux-rbs-rfp-Ter, Prlux-rbs-rfp-rbs-luxI, Prlux-rbs-rfp-rbs-luxI-Ter, Prlux-rbs-rfp-rbs-luxR-Ter, Prlux-rbs-rfp-luxR, Prlux-rbs-rfp-luxI-rbs-luR, Prlux-rbs-rfp-rbs-luxI-rbs-luxR-Ter.

Sequencing results:

Prlux-rbs-rfp-T:

TATAAaAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAA
ATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTT
CTAGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTG
TTATAGTCGAATAAATACTAGAAAAGAGGAGAAAATGGTGAGCAAG
GGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTC
AAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGAT
CGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACC
GCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTG
GGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGT
GAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCC
CGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCG

GCGTGGTGACCGTGACCCAGGACTCCTCCTTGCAGGACGGCGAG
TTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGAC
GGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTC
CGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCA
AGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAG
GTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGG
CGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGA
GGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCC
ACTCCACCGGCGGCATGGACGAGCTGTACAAGTAATAATACTAGA
GCCAGGCATCAAATAAAACAAAAGGCTCAGTCGAAAGACTGGGCC
TTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCA
CACTGGTTCACCTTCGGGTGGGCCTTTCTGCGTTTATAGAATTCAC
TAGTGCGGCCGCTGCAGTCCGGCAAAAAAGGGCAAGGTGTCACC
ACCCTGCCCTTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGC
AGGCTTCCTCGCTCACTGACTCGCTGCGCTCGgTCGT

Prlux-rbs-rfp-rbs-luxI-T:

aTTAACCTATAAAAATAGGCGTATCACGAGGCAGAATTTCAGAT
AAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCG
GCCGCTTCTAGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAA
ATGGTTTGTTATAGTCGAATAAATACTAGAAAAGAGGAGAAAATGGT
GAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCAT
GCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGT
TCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCAC
CCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCT
TCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGG
CCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGT
CCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAG
GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCTTGCAGGA
CGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCC
CCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAG
GCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGG
GCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTAC
GACGCTGAGGTCAAGACCACCTACAAgGCCAAGAAGCCCGTGCA
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AGCAATAGAGCGATTTTTAAAGCGTATTAAAGTTCCTTGTCATCGTA
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TGCAAACGACGAAAACTACGCTTTAGTAGCTTAATAACTCTGATAGT
GCTAGTGTAGATCTCTAATAATACTAGAGCCAGGCATCAAATAAAAC
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CCGCTGCAGTCCGGCAAAAAAGGGCAAGGTGTCACCACCCTGCC
CTTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCAGGCTTCC
TCGCTCACTGACTCGCTGCGcTCGgTCgtT

Prlux-rbs-rfp-rbs-luxR-T:

GGCGTATCACGAGGCAGAATTTCAGATAAAAAAAATCCTTAGC
TTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTAGAGACC
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CATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCG
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GAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCC
TGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACC
CCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCT
TCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTG
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ATAACACTGATAGTGCTAGTGTAGATCACTAATAATACTAGAGCCAG
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GTCACCACCCTGCCCTTTTTCTTTAAAACCGAAAAGATTACTTCGC
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Prlux-rbs-rfp-rbs-luxI-rbs-luxR-Ter

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GTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCG
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GGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAG
ACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTA
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CAAAAAAGGGCAAGGTGTCACCACCCTGCCCTTTTTCTTTAAAACC
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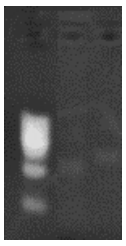
CTGCGC

E. Linking of Pcons2-rbs-lacI-T and Plac-rbs-luxI-T



From left to right, Marker(1Kb), Pcons2-rbs-lacI-Ter, Pcons2-rbs-lacI-Ter-Plac-rbs-luxI-Ter

Sequencing results:

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AATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCT
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CTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATG
CGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTC
GGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCG
CCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACC
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GGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAAC
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ACAATTTCACACATACTAGAGAAAGAGGAGAAATTAAGCATGACTAT
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CATCAACAGCAATAGAGCGATTTTTAAAGCGTATTAAAGTTCCTTGT
CATCGTATTGGAGACAAAGAAATTCATGTATTAGGTGATACTAAATC
GGTTGTATTGTCTATGCCTATTAATGAACAGTTTAAAAAAGCAGTCT

TAAATGCTGCAAACGACGAAAACTACGCTTTAGTAGCTTAATAACTC
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GTTGTTTGTCGGTGAACGCTCTCCACTCGGTCACACTGAGACCTA
GTAGCGGCCGCTGCAGTCCGGCAAAAAAGGGCAAGGTGTCACCA
CCCTGCCCTTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCA
GGCTTCCTCGCTCACTGACTCGCTGCGCTCgGTCG

F. Linking of Pcons2-rbs-lacI-T-Plac-rbs-luxI-T and Pcons2-rbs-luxR-T

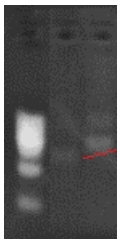


Figure: From left to right, Marker(1Kb), Pcons2-rbs-lacI-T-Plac-rbs-luxI-T, Pcons2-rbs-lacI-T-Plac-rbs-luxI-T-Pcons2-rbs-luxR-Ter.

G. Completion of four systems.

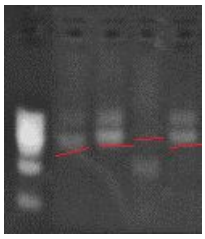
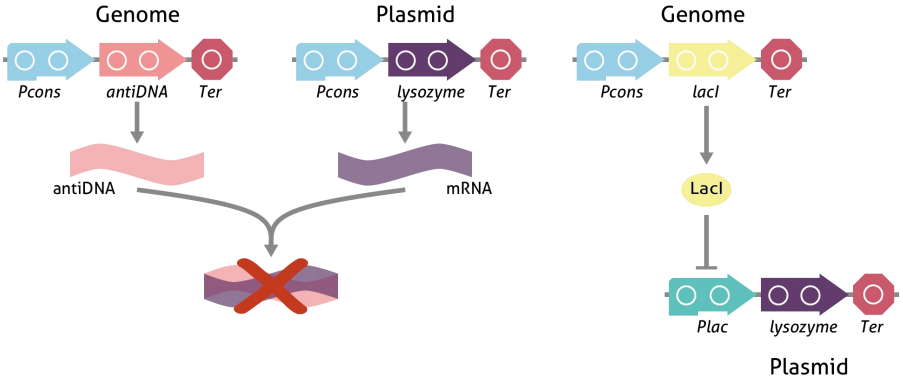


Figure: From left to right, Marker(1Kb), Pcons2-rbs-lacI-T-Plac-rbs-luxI-T-Pcons2-rbs-luxR-Ter, Pcons2-rbs-lacI-T-Plac-rbs-luxI-T-Pcons2-rbs-luxR-Ter-Prlux-rbs-rfp-Ter, Pcons2-rbs-lacI-T-Plac-rbs-luxI-T-Pcons2-rbs-luxR-Ter-Prlux-rbs-rfp-rbs-luxI-Ter, Pcons2-rbs-lacI-T-Plac-rbs-luxI-T-Pcons2-rbs-luxR-Ter-Prlux-rbs-rfp-rbs-luxI-rbs-luxR-Ter.

(2) Testing of function

3. Suicide circuit



(1) Construction of plasmid

A. Division 1 PgolTS-golS-PgolB-rbs-tetR-Ter

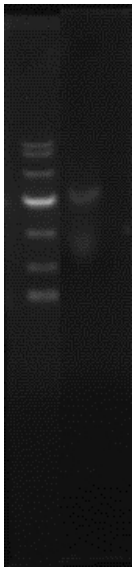


Figure: From left to right, Marker(DL10000), PgolTS-golS-PgolB-rbs-tetR-Ter

Sequencing results:

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ATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTA
GAGACAACGAAGGAAAGGTCAAGCGTTCCTGACGGGTTTTTACGGGG
CGTGGGTCGGCATCGTGGCGTAAATGTCTCGCATCATCCTCTTTTATGA
GCCATCTCACATTCTCGCCGAACCGTGCAGCCTGAATACGCTTGACCT
TCCCACAATGGCAAGCTTCAGGCTTTCTGATACCGAATAGTCAGGATG
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CATCGAAAGTCTCGGCCAAAATGATTCGCTACTATGAACAGATTGGTCT
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GGCTGATGTTAATCAATTGCATTTTATACGCCGCGCGCGCGACCTCGGT
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CGTCTGTAAGTCCTGAGATTACGCTTGACCTTCCAACACTGGCAAGGT
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GCTCTCCACTCGGTCACACTGAGAACTAGTAGCGGCCGCTGCAGTCC
GGCAAAAAAGGGCAAGGTGTCACCACCCTGCCCTTTTTCTTTAAAACC
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GCGCTCGGTCGTtCGG

B. Division 2 Ptet-rbs-rfp-T:

Figure: From left to right, Marker（DL2000）, Ptet, Ptet-rbs-rfp, Ptet-rbs-rfp-T. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

Sequencing results:

ACCTATAAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAA
ATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTA
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TGAGCACTACTAGAAAAGAGGAGAAAATGGTGAGCAAGGGCGAGGAG
GATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATG
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GGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAG
GGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACG
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CTCCACCGGCGGCATGGACGAGCTGTACAAGTAATAATACTAGAGCCA
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AGTAGCGGCCGCTGCAGTCCGGCAAAAAAGGGCAAGGTGTCACCACC
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C. Division 3 Plac-rbs-tetR-T:

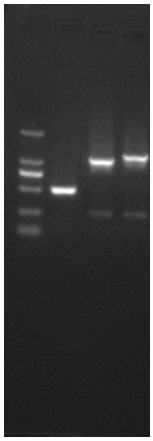

Figure: From left to right, Marker （ DL2000 ） , Plac, Plac-rbs-tetR, Plac-rbs-tetR-T. The substances of electrophoresis are made from PCR of plasmid with VF2 and VR as their primers.

Sequencing results:

GGCGTATCACGAGGCAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGA
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CCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGG
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GACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGC
TTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCT
GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACT
ATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCcACTGGCAGCAGCCACTG
GTAaCAgGATAGCAGAGCGAGTATGTAGGCGGTGCTACGAGTTCTTGATGGTGCTTA
CTACGGCTACCTAGAAGAACAGTAAT

D. Division 4 Pcons-rbs-lacI-T:

The figure is the same with that in the positive feedback circuit.

Sequencing results:

It is the same with that in the positive feedback circuit.

E. Linking of PgolTS-golS-PgolB-rbs-tetR-Ter and Ptet-rbs-rfp-Ter

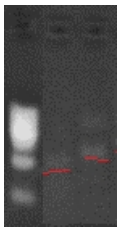


Figure: From left to right, Marker(1Kb), PgolTS-golB-PgolB-tetR-Ter, PgolTS-golB-PgolB-tetR-Ter-Ptet-rbs-rfp-Ter.

F. Linking of PgolTS-golS-PgolB-rbs-tetR-Ter-Ptet-rbs-rfp-T and Plac-rbs-tetR-T

G. Linking of PgolTS-golS-PgolB-rbs-tetR-Ter-Ptet-rbs-rfp-T-Plac-rbs-tetR-T and Pcons2-rbs-lacI-T

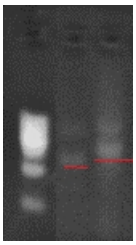


Figure: From left to right, Marker(1Kb), PgolTS-golB-PgolB-tetR-Ter-Ptet-rbs-rfp-Ter, PgolTS-golB-PgolB-tetR-Ter-Ptet-rbs-rfp-Ter-Plac-rbs-tetR-Ter-Pcons2-rbs-lacI-Ter.

(2) Testing of function

A. The test of function of Ptet-rbs-rfp-Ter shows that without tetR, rfp was expressed.

The left one is Ptet-rbs-rfp-Ter, and the right one is another plasmid. The photo shows that without tetR, rfp will be produced, and bacterial will get red.

4. OMPA-golB:

Figure: From left to right, Marker(DL10000), OMPA-golB.

Sequencing results:

TAATAAAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAAATCC
TTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTAGAGAAAG
AGGAGAAATTAAGCATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGG
CTGGTTTCGCTACCGTAGCGCAGGCCGCTCCGAAAGATAACACCTGGTACA
CTGGTGCTAAACTGGGCTGGTCCCAGTACCATGACACTGGTTTCATCAACA
ACAATGGCCCGACCCATGAAAACCAACTGGGCGCTGGTGCTTTTGGTGGTT
ACCAGGTTAACCCGTATGTTGGCTTTGAAATGGGTTACGACTGGTTAGGTC
GTATGCCGTACAAAGGCAGCGTTGAAAACGGTGCATACAAAGCTCAGGGC
GTTCAACTGACCGCTAAACTGGGTTACCCAATCACTGACGACCTGGACATC
TACACTCGTCTGGGTGGCATGGTATGGCGTGCAGACACTAAATCCAACGTT
TATGGTAAAAACCACGACACCGGCGTTTCTCCGGTCTTCGCTGGCGGTGTT
GAGTACGCGATCACTCCTGAAATCGCTACCCGTCTGGAATACCAGTGGACC
AACAACATCGGTGACGCACACACCATCGGCACTCGTCCGGACAACGGAGT
CGACATGCAGTTCCATATTGATGACATGACCTGCGGCGGCTGCGCCAGTAC
GGTAAAAAAGACGATTCTGACTCTCGATGCTAATGCGACGGTGAGAACTGA
CCCGGCGACGCGTCTGGTTGACGTTGAAACGTCGCTATCCGCGGAGCAGA
TTGCCGCCGCCCTGCAAAAGGCCGGTTTCCCGCCGCGCGAGAGGCTCGA
GGATTACAAGGATGACGACGATAAGATCTAATAATACTAGTAGCGGCCGCTG
CAGTCCGGCAAAAAAGGGCAAGGTGTCACCACCCTGCCCTTTTTCTTTAAA
ACCGAAAAGATTACTTCGCGTTATGCAGGCTTCCTCGCTCACTGACTCGCT
GCGCTCGTC

5. MlrD and MrlA

(1) Construction of plasmid

MlrD:

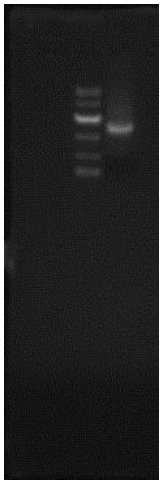


Figure: From left to right, Marker(DL10000), MlrD.

Sequencing result:

GATCaACCAGCGGTCTTACTGCTGGCTGCGACGGAAATGTGGGAGGC
CTTTTCCTATGTCGGGCTCAGAACCGTACTGGTCTACTACCTTACGCA
GGACCTGGGCTATTCGACCGAGGACGCCTCACTTATCTATGGGACGT
TCCTCGGCGTAGCCTATGTAACGCCAATCCTCGGTGGGTGGATCGCC
GATAGGTTTATTGGCCGTTCGGCGGCAATTGTCGGTGGCGCATTGCT
GAAGATGGCCGGGTACATCGGCCTTTTGCTTGGCGCGAACGTTACGG
GCTGCCTCGCCGCAATTGTCATTGGCAATGGCCTGTTTCTTCCAACTC
TGCCCGCTACGCTGGGTGCTCTTTTCTCGCCGAACGACCCCGATCGC
CAGCGCAGTTTCAGCTTCTACTATCTCGCAGTGAGCGCTGGTGCGCT
GCTGGCACCGCTGATCTGCGGCACGCTTGGAGAGAATTTCGGCTGG
CGGTACAGTTTCCTCGCTTCCGCTACCGGACTTGCAGCAGCCATCGT
TATCTTTCTCGCCGGACGCCATCTTCTGCCTCCAGACCGACCTGGAG
CAGCGTCCCGTCTGGTCGACGAAACGCCGGTCGCGCAGGCGAGCC
AGTCCGTCATCCCGCTCCTGGCAGGTGTCCTTGCAGCAGTAATCGTC
TTGCGGGTCGCTTATGAGCAACTCGGCAACACTGTCGCGCTTTTCGC
CGCCAGCCAGGTCGATCGTTCGCTAGGGGCAGATATCACCATCCCTT
ATACCTGGTTTCAGTCGCTCAATCCGCTGGGGGTCATTCTGTTCACCC
CGCTCCTCGTCTGGGGCTGGCGTAAGGCCGCCGCGAGAGGTGGCT
CGCAGAACGACTATTTCAGGATGGCCATTGGTAGCTGCATCATGGCC
GCGGCCTTTGTTGGGCTCGCTCTGATCATCCATCTCGGGCAACCCGG
TCAGATTTTCTGGCCTGTTCTAGCAGCGTTTTTTCTGAtGGTAACCTTT
GGCGAGTTGTGGGTGCTTCCGGTCGGTCTCAGTCTGTTTGCGCGCTT
GGCACCCGCAGGGCGAGGTGCAGTCACCATCGCGTTCTGGTATAGC
GCGCGAGCCGCTGGCAATTTTCTTGCCGGATTGATGGGGCGCGCCG
AACCTGCACTCGGATATGGCAACTTCTTCCTGCTTTGCGCAGTGTTTC
CGCTGCTGGCAGCAACGATCTTCGTCGCGATCGGCAAGCGCTCGC

(2) Testing of function

6. Attachment

(1) Complete photos of electrophoresis

A.

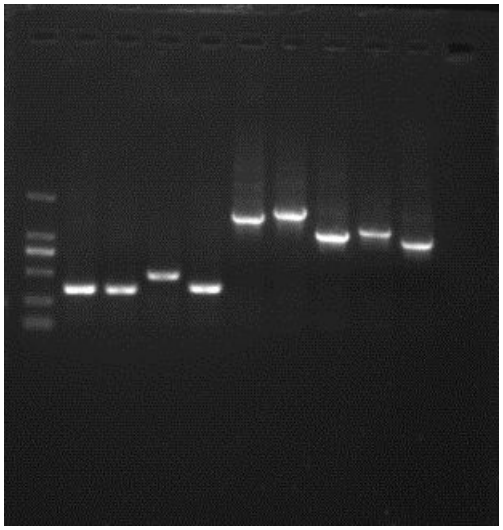


Figure: From left to right, Marker(DL2000),Pcons,Pcons-rbs-luxR,Pcons-rbs-luxR-T,Prlux,Prlux-rbs-lacI,Prlux-rbs-lacI-T,Prlux-rbs-CI,Prlux-rbs-CI-T,Prlux-rbs-rfp. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

B.

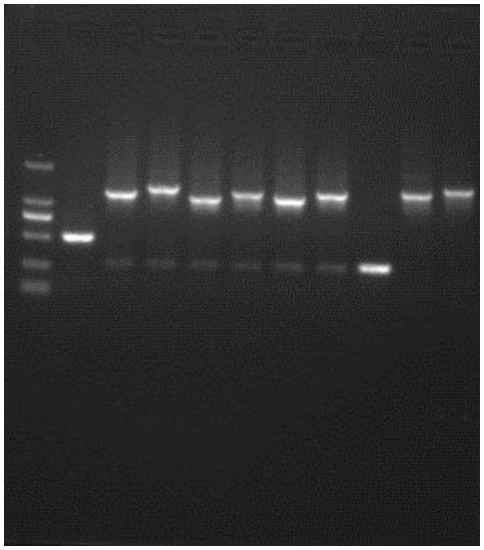


Figure: From left to right, Marker(DL2000),Plac,Plac-rbs-rfp,Plac-rbs-rfp-T,Plac-rbs-luxI,Plac-rbs-luxI-T,Plac-rbs-tetR,Plac-rbs-tetR-T,PCI,PCI-rbs-lacI,PCI-rbs-lacI-T

. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

C.

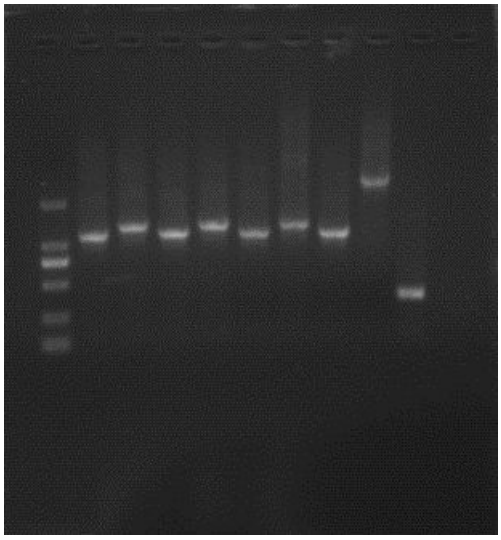


Figure: From left to right, Marker(DL2000) Prlux-rbs-rfp, Prlux-rbs-rfp-T, Prlux-rbs-rfp-rbs-luxI, Prlux-rbs-rfp-rbs-luxI-T, Prlux-rbs-rfp-rbs-luxR, Prlux-rbs-rfp-rbs-luxR-T, Prlux-rbs-rfp-rbs-luxI-rbs-luxR,Prlux-rbs-CI-T-PCI-rbs-lacI-T,Ptet. The substances of electrophoresis are made from PCR of plasmids with VF2 and VR as their primers.

D.

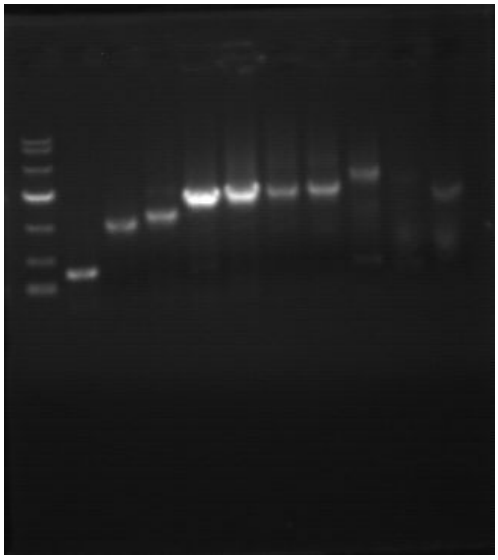


Figure: From left to right, Marker(DL10000), Prlux, Prlux-rbs-rfp, Prlux-rbs-rfp-Ter, Prlux-rbs-rfp-luxI, Prlux-rbs-rfp-rbs-luxI-Ter, Prlux-rbs-rfp-rbs-luxR, Prlux-rbs-rfp-rbs-luxR-Ter, Prlux-rbs-rfp-rbs-luxI-rbs-luxR, Prlux-rbs-rfp-rbs-luxI-rbs-luxR-Ter, PgolTS-golS-PgolB-rbs-tetR-Ter.

E.

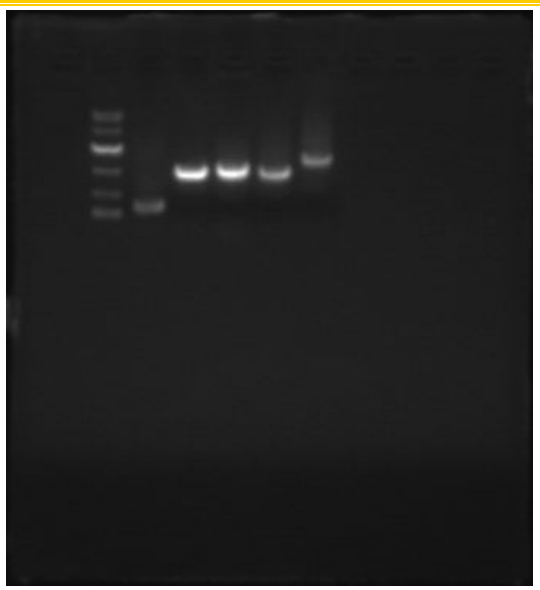


Figure: From left to right, Marker(DL10000), Ptet, Ptet-rbs-rfp, Ptet-rbs-rfp-Ter, OMPA-golB, MlrD.

F.

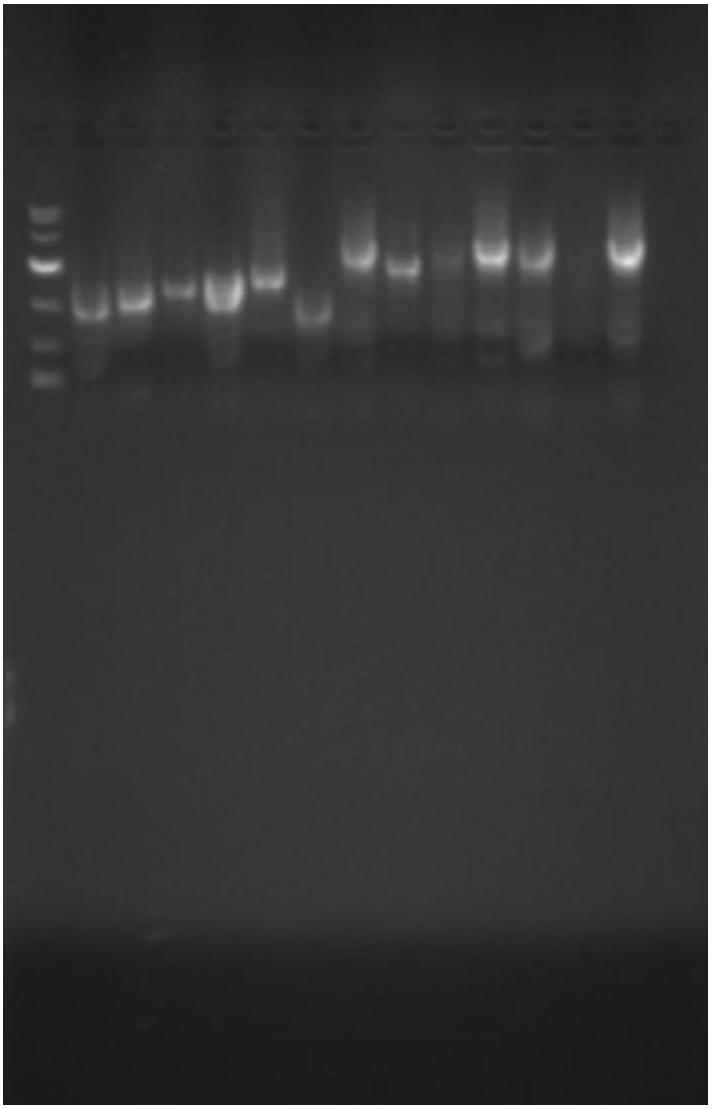


Figure:From left to right, Marker (DL10000), Pcons2, Pcons2-rbs-luxR, Pcons2-rbs-luxR-Ter, Pcons2-rbs-lacI, Pcons2-rbs-lacI-Ter, Prlux-rbs-rfp, Prlux-rbs-rfp-rbs-luxR-Ter, Prlux-rbs-rfp-rbs-luxR, Prlux-rbs-rfp-rbs-luxI-rbs-luxR, Prlux-rbs-rfp-rbs-luxI-rbs-luxR, Prlux-rbs-rfp-rbs-luxI-rbs-luxR, Prlux-rbs-rfp-rbs-luxI-rbs-luxR, Prlux-rbs-rfp-rbs-luxI-rbs-luxR-Ter, Prlux-rbs-rfp-rbs-luxI-rbs-luxR-Ter.

G.

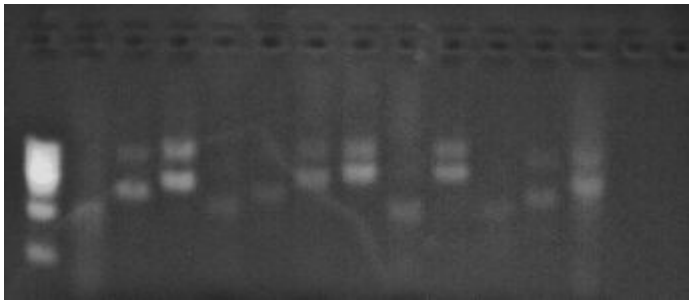
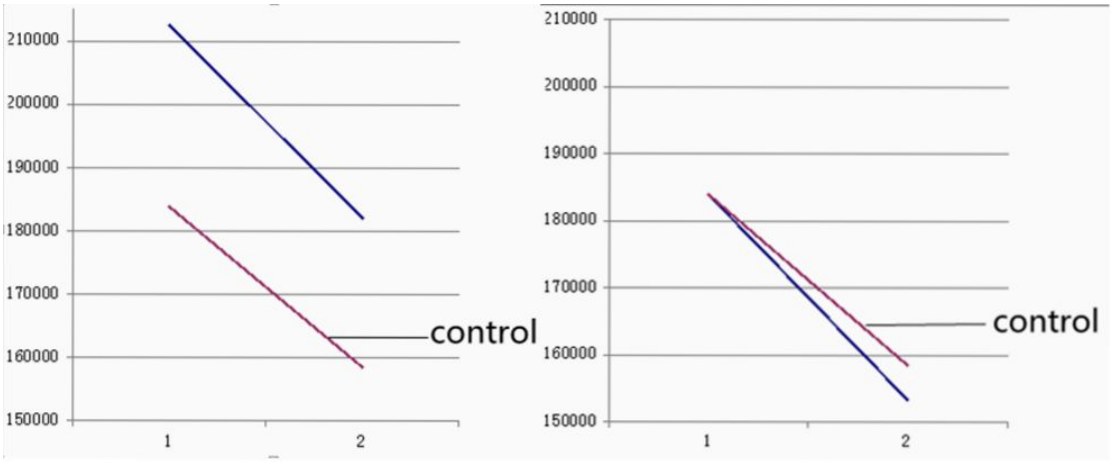


Figure: From left to right, Marker(1Kb), Pcons-rbs-luxR-Ter, Pcons-rbs-luxR-Ter-Prlux-rbs-lacI-Ter, Pcons-rbs-luxR-Ter-Prlux-rbs-lacI-Ter-Plac-rbs-rfp-Ter, Pcons-rbs-lacI-Ter, Pcons-rbs-lacI-Ter-Plac-rbs-luxI-Ter, Pcons-rbs-lacI-Ter-Plac-rbs-luxI-Ter-Pcons-rbs-luxR-Ter, Pcons-rbs-lacI-Ter-Plac-rbs-luxI-Ter-Pcons-rbs-luxR-Ter-Prlux-rbs-rfp-Ter, Pcons-rbs-lacI-Ter-Plac-rbs-luxI-Ter-Pcons-rbs-luxR-Ter-Prlux-rbs-rfp-rbs-luxI-Ter, Pcons-rbs-lacI-Ter-Plac-rbs-luxI-Ter-Pcons-rbs-luxR-Ter-Prlux-rbs-rfp-rbs-luxI-rbs-luxR-Ter, PgolTS-golB-PgolB-rbs-tetR-Ter, PgolTS-golB-PgolB-rbs-tetR-Ter-Ptet-rbs-rfp-Ter, PgolTS-golB-PgolB-rbs-tetR-Ter-Ptet-rbs-rfp-Ter-Pcons-rbs-lacI-Ter.

Part 2:HPLC



The curve above respresents our bacteria with plasmid.(left)
To compare the slope of thecurves, we move the one above(right)

From the HPLC result, we can conclude that express MlrD in bacteria can increase their MC absorbing ability. Although the concentrations of microcystin in two treatments at 0h

are different, the decrease of MC in the first hour is faster in MlrD bacteria than it in the control (bacteria without MlrD). The difference at 0h can be explained by the fact that IPTG can kill some of the bacteria, which makes the number of bacteria in the IPTG (+) tube is less than the number in the control tube, and that can result in the absorbing quantity of MlrD bacteria lower than control one at the beginning (So the concentration of MC in this tube is higher at 0h). This fact can also explain the phenomenon that there is a decrease of MC concentration in the control group during the one hour cultivation.